Variation and Breeding of Kikuyu Grass (*Pennisetum clandestinum*)

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Declaration of Originality

The contents of this thesis are, to the best of my knowledge, entirely my own work except where otherwise attributed. This material has not been submitted previously to this University or any other higher education institution for any degree or diploma.

Brett W. Morris
10th December, 2009

Dedication

To Kellie and Charlotte

For your love, support, understanding and encouragement,

For without you I would not be where I am today.

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Abstract

This study examined the variation existing in naturalised populations of kikuyu grass (*Pennisetum clandestinum* Hochst. ex. Chiov) in Australia, as well as initiating a breeding programme aimed at producing new hybrid lines for the Australian turfgrass and agricultural market. The first part of the study examines the phenotypic variation which exists within kikuyu grass populations; the genotypic variation of those populations via DNA marking; and, the basis of male sterility within those populations. The second part examines kikuyu grass within a breeding perspective through pollen viability and storage; the potential presence of an endophyte within the seed; classical hybridisation of ecotypes through to field planting; and, whether the oomycete *Verrucalvus flavofaciens* can be controlled via a modern day fungicide programme. It also rewrites the history of kikuyu introduction, first seeding occurrence, and previously unrecorded importations into Australia. General observations record the first photographic images of kikuyu grass chromosomes.

Significant phenotypic variation exists within naturalised kikuyu grass populations across Australia. From a collection of about 200 ecotypes 16 were selected for detailed study. Analysis of the ecotypes identified two lines from several which show great potential within the Australian turfgrass and agricultural market; the first selected at Grafton, NSW, which in the leaf width analysis displayed a leaf width over 18% finer than the mean; with the second selected at Morphettville, SA, which in the stolon width analysis displayed a stolon width over 15% thicker than the mean. Both selections, as well as others, displayed positive traits which would appeal to a wide range of end users.

Genetic investigations using RAPD marker techniques are undertaken on kikuyu for the first time. A total of thirteen decamer primers produced 195 markers of which 93.85% were polymorphic. Genotypic variation amongst the Australian selections was found to range from 28.8% - 82.4%. Relatedness between the cluster accessions used in the phenotypic analysis and the dendogram produced in the genetic analysis was not found.

Male sterility within Australian kikuyu grass was determined to exist as a recessive condition. From the F_1 population, 100% transformation from male sterile to fully fertile was observed; with the F_2 population segregating into a 52.5% fully fertile, 47.5% male sterile. Negative interactions between parental lines were observed.

Kikuyu grass pollen is most viable in the first few hours after shedding, and deteriorates significantly within 24 hours, even at low temperatures, if it is stored. Pollen viability varies amongst genotypes. Prior additions of dry colloidal material does not assist in storage capabilities.

Investigations into seedling mortality of kikuyu identified the possible presence of an endophyte within the seed. Surface sterilisation techniques provided no control, with an addition of 0.1% PPM to the base agar mixture the most effective form of control. Intercellular hyphae were identified and photographed after staining with Rose Bengal.

Hybridisation studies of kikuyu grass resulted in several potential lines worthy of continued analysis. Selections from varying growing environments around Australia were hybridised with three pollen parents derived from chemical mutagenesis producing a total of 349 hybrid F_2 seeds. Germination and screening in the glasshouse resulted in 14 hybrid lines being field planted alongside cv. 'Whittet' for comparison. The opportunity exists within the turfgrass market for elite lines of kikuyu, which will cover a wide range of uses from golf course tees and fairways, sporting grounds and race tracks, to pasture and commercial use.

Efficacy with modern day fungicides *in vitro* was found not successful in controlling Kikuyu Yellows (*Verrucalvus flavofaciens*). Resistance of kikuyu grass to the oomycete will have to come in the form of genetically resistant cultivars; production of a specific fungicide; or both.

Table of Contents

Attached CD:

List of Tables

List of Figures

Appendices

List of Abbreviations

ASL:	Above sea level
bp:	Base pair
°C:	Degrees Celsius
Ca:	Calcium
$CaNO_3$:	Calcium Nitrate
cal:	Calorie
cm:	Centimetre
CMS:	Cytoplasmic Male Sterility
CP:	Crude Protein
Cu:	Copper
d:	Day
DM:	Dry matter
DNA:	Deoxyribonucleic Acid
EC:	Electrical Conductivity
Fe:	Iron
ft:	Feet
GDR:	Great Dividing Range
g:	Gram
g/L:	Grams per litre
h:	Hour
Ha:	Hectare
HAC:	Hawkesbury Agricultural College
H_3BO_3:	Boric Acid
K:	Potassium
KC:	Kikuyu Collection
kg:	Kilogram
KH:	Kikuyu Hybrid
km:	Kilometre
KNO_3:	Potassium Nitrate
m:	Metre
Mg:	Magnesium
$MgCl_2$:	Magnesium Chloride
$MgSO_4$:	Magnesium Sulphate
mm:	Millimetre
mM:	Millimolar
Mn:	Manganese
Mo:	Molybdenum
N:	Nitrogen
Na:	Sodium
NaOCl:	Sodium Hypochlorite
ng:	Nanogram
NSW:	New South Wales
P:	Phosphorus
PBI:	Plant Breeding Institute
PCR:	Polymerase Chain Reaction
ppm:	Parts per million
QLD:	Queensland

RAPD:	Random Amplified Polymorphic DNA
rpm:	Revolutions per minute
rps:	Revolutions per second
S:	Sulphur
SA:	South Australia
sec:	Seconds
t:	Tonne
TAS:	Tasmania
U:	Unit
UV:	Ultraviolet
V:	Volts
VIC:	Victoria
WA:	Western Australia
Zn:	Zinc
%	Percent
-	Minus
£	Pound Currency
µL	Microlitre
µM	Micromolar